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TI Ablating the ischemia-reperfusion injury in non-heart-beating donor kidneys.
AU Hernandez A; Light J A; Barhyte D Y; Mabudian M; Gage F look for definition of Belzer MPS
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TI Optimal pH for simple cold storage or machine perfusion of dog kidneys with UW solution.
AU Lindell S; Nobel M; Rankin M; D'Alessandro A; Southard J H
SO TRANSPLANT INTERNATIONAL, (1998) 11 (3) 208-11.

AN 95291541 MEDLINE
DN 95291541 PubMed ID: 7773485
TI Prostaglandin E1 attenuation of ischemic renal reperfusion injury in the rat. look for concentration of PGE1
AU Vargas A V; Krishnamurthi V; Masih R; Robinson A V; Schulak J A
SO JOURNAL OF THE AMERICAN COLLEGE OF SURGEONS, (1995 Jun) 180 (6) 713-7.

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ORIGINAL ARTICLE

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Optimal pH for simple cold storage or machine perfusion of dog kidneys with UW solution

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Abstract Metabolic suppression by temperature is a key to successful organ preservation. Additional methods for inducing metabolic suppression may further improve organ preservation. Extracellular acidosis has been shown to suppress warm anoxic injury to various isolated cells. Acidosis may suppress enzymes with a pH optimum at the pH of the cytosol (pH 7.3). In this study, the combination of hypothermia and acidosis was used to determine if it would improve renal preservation. Dog kidneys were cold-stored (CS) for 48 h in University of Wisconsin (UW) solution with the pH adjusted to 6.4, 6.8, 7.4, or 7.8. Kidneys were also machine-perfused (MP) for 3 days with the gluconate perfusion solution (Belzer's machine perfusion solution, MPS) at pHs similar to those tested for CS. Renal function (serum creatinine, SCr) and survival were recorded in immediate contralateral nephrectomized recipients. On the basis of maximum SCr values, kidneys preserved by CS or MP were best preserved at pHs of 7.4 or 7.8. At a pH of 6.8, SCr values were elevated and returned to normal at a slower rate than in those preserved at higher pHs. This study shows that acidosis is not cytoprotective to cold-stored dog kidneys and causes preservation/reperfusion injury.

Key words Kidney preservation, UW solution, cold storage · pH, machine perfusion, kidney · Cold storage, pH, kidney · Preservation, kidney, pH

Introduction

One of the most important factors in organ preservation is metabolic suppression by hypothermia. At temperatures commonly used to cold-store organs (0°–4°C), enzyme reaction rates are slowed about 10- to 13-fold [8]. Although slowed, enzyme catalysis continues and in long-term storage the activity of hydrolytic enzymes (proteases, phospholipases, etc.) may cause injury to the tissue, leading to organ failure on transplantation [9, 11, 13]. Thus, methods that suppress metabolism, in addition to hypothermia, may contribute to improved organ preservation.

The activity of hydrolytic enzymes is pH-dependent and many have a pH optimum near the pH of the cytosol (about 7.3). Lowering the pH is a way of suppressing the activity of these enzymes and, in combination with hypothermia, could be an effective method for extending the quality and duration of cold-stored organs. The use of acidic conditions to prevent tissue injury from hypoxia is not a new idea; Pentilla and Trump [15] used acidic conditions to protect rat renal cells from anoxia. Bonventre and Cheung [2] found similar results in renal tissue, and Lemasters' group used low pH to suppress hypoxic injury in liver [4, 10] and cardiac cells [1]. These studies, although done at normothermia, suggest that acidic preservation conditions (cold anoxia) may be beneficial to long-term organ storage.

Our goal in this study was to determine if lowering the pH of UW solution (used for simple cold storage) or of the perfusion solution (Belzer's MPS, used for continuous machine perfusion) would improve efficacy in

dog kidney preservation. Dog kidneys were cold-stored or machine-perfused for 2 or 3 days in UW solution with the pH adjusted to 6.4, 6.8, 7.4, or 7.8.

Materials and methods

Beagle dogs weighing 12–15 kg were used. "Principles of Laboratory Animal Care" (NIH publication no. 86–23, revised 1985) were followed. Kidneys were removed and preserved by methods commonly used in our laboratory [12]. For simple cold storage, the kidney was flushed through the renal artery with 150 ml of cold (4 °C) UW solution and stored at 5 °C. For machine perfusion, the kidney was flushed with 150 ml cold (4 °C) saline and perfused on a mini-Belzer perfusion machine at an initial pressure of 50 mm Hg (systolic) at 5 °C. The perfusate (UW gluconate) [14] was allowed to equilibrate with room air and the pO_2 was about 120–150 mm Hg. Perfusion pressure decreased during the 1st h of perfusion to about 40 mm Hg and remained at this pressure for the duration of the experiment.

The preservation solutions were modified to achieve the desired pH at room temperature. The pH was adjusted with HCl or NaOH for the perfusate and with KOH for UW solution. Once adjusted, the pH was maintained during the period of preservation. After preservation, the kidneys were transplanted (autotransplant); this was followed by an immediate contralateral nephrectomy. Serum creatinine (SCr) values were measured on a Kodak Ektachem clinical analyzer on a daily basis. The number of kidneys preserved and transplanted at each pH is given in the legend to Figs. 1 and 2, along with the preservation times.

Results

Simple cold storage

A previous study showed that UW solution effectively preserved mongrel dog kidneys for 72 h with 100% survival and only minimal injury (SCr rose to about 5 mg/dl by day 3 post-transplant) and a return to normal SCr (about 1.0–1.5 mg/dl) by day 7 [16]. In this study, we used beagles and obtained similar results. After 3 days of cold storage with UW solution (pH 7.4), we had 100% survival (5 of 5), peak SCr (5.5 ± 1.8 mg/dl) on day 4, and normal SCr (1.7 ± 0.5 mg/dl) by day 10.

To compare UW solution at different pHs, however, we cold-stored dog kidneys for only 2 days because, clinically, kidneys are preserved for only 24–30 h on average and very few require more than 48 h of preservation. Kidneys preserved at pH 7.4 for 2 days showed only a small degree of functional injury; SCr rose to 2.9 ± 0.9 mg/dl on day 2 and returned to normal by day 10 (Fig. 1). Similar results were obtained at pH 7.8. Greater renal functional injury was obtained by storage at lower pHs (6.4 or 6.8). All kidneys stored at pH 6.8 survived (5 of 5) but had delayed graft function (Fig. 1). Kidneys preserved at pH 6.4 were not viable, and we sacrificed both dogs on the 6th postoperative day. Only two dogs were placed in this group since it was evident

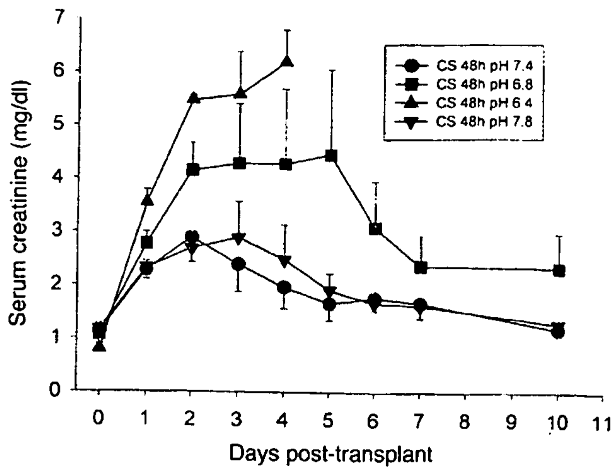

Fig. 1 Effect of pH of UW solution on 48-h cold storage (*CS*) of dog kidneys. Dog kidneys were cold-stored in UW solution for 48 h and transplanted with immediate contralateral nephrectomy. Serum creatinine values were measured daily and are reported as means with standard error of the means represented by *vertical lines*. The number of dogs in each group is as follows: pH 7.4 = 4, pH 7.8 = 4, pH 6.8 = 4, and pH 6.4 = 2

in these two experiments that pH 6.4 was not a useful pH for renal preservation. Furthermore, our policy is to reduce the use of animals in experimental research by not conducting unnecessary experiments.

The poorer quality preservation at pH 6.8 may have been due to the abrupt change in pH after transplantation and to the exposure of the renal vasculature to blood at pH 7.4, as suggested in studies by Lemasters et al. in rat liver transplantation [7]. This group [6] developed a method for reducing injury caused by the abrupt change in pH by flushing the liver with a cold acidic solution prior to transplantation. To test the effect of a cold acid flush, we flushed out the renal vasculature of pH 6.8 cold-stored kidneys ($n = 3$) with saline (pH adjusted to 6.8). In these three dog kidney transplants, all dogs survived but renal function was poor. SCr was elevated to 6.0 ± 1.0 mg/dl on day 3 and remained high (> 3.5 mg/dl) for 1 week. This result was worse than in kidneys preserved at 6.8 and transplanted without a pretransplant vasculature flushout. The small numbers; however, prevent a detailed analysis of this data in a meaningful way.

Machine perfusion

Dog kidneys machine-perfused for 3 days with a perfusate at pH of 7.8 or 7.4 yielded excellent transplant results and minimal renal injury (Fig. 2). SCr values increased to only about 2.4 mg/dl on post-transplant days 1 and 2, followed by a return to normal values. Kidneys

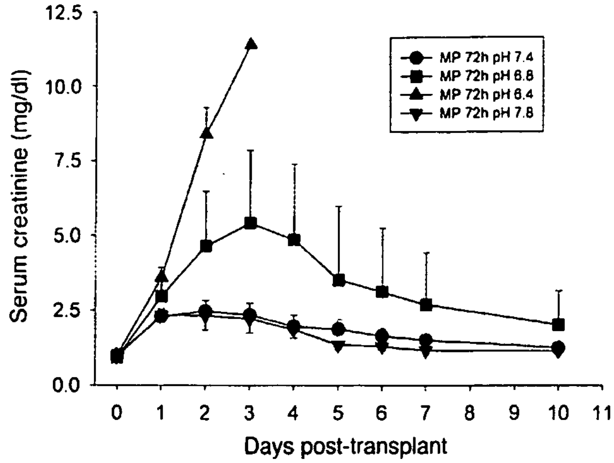

Fig. 2 Effect of pH of Belzers MPS on 72-h machine perfusion (*MP*) of dog kidneys. Dog kidneys were continuously perfused for 72 h and transplanted. Serum creatinine values were measured daily and are reported as means with standard error of the means represented by *vertical lines*. The number of dogs in each group is as follows: pH 7.4 = 4, pH 7.8 = 4, pH 6.8 = 4, and pH 6.4 = 2

machine-perfused at pH 6.8 or 6.4 did worse than those preserved at 7.4 or 7.8. Both dogs receiving a kidney preserved at 6.4 were sacrificed on the 5th day due to renal failure. All kidneys preserved at 6.8 (4 of 4) survived but with functional injury, evident by the elevated SCr values and slow return of normal renal function (Fig. 2).

Discussion

Cold storage of kidneys is a highly successful method for preserving the organ prior to transplantation, and 1-month and 1-year graft survival rates are 90% or more [5]. Improvements in organ preservation are, however, necessary because 20%–30% of cadaver kidneys preserved by cold storage have delayed function (i.e., need for post-transplant dialysis because of poor recovery of renal function). This is costly, hinders the optimization of immunosuppressive therapy, and may be a cause of chronic rejection and late graft loss. Furthermore, there is an increasing interest in expanding the number of kidney donors to include the nonheart-beating donor. Kidneys from this group of donors could decrease the current organ shortage, but good quality preservation of these organs is more difficult because the pre-existing hypotensive or ischemic injury is additive to the cold ischemic damage. Kidneys from nonheart-beating cadavers are preserved better by machine perfusion than cold storage [3]; however, they often have delayed graft function. To decrease delayed graft function and expand the donor pool may require better methods of preservation of the nonideal kidney.

In the past, we have made many attempts to improve upon UW solution but we have not tested how pH affects kidney storage. In this study, we show that the pH of UW solution or of the perfusion solution (pH 7.4) appears ideal for both simple cold storage and machine perfusion. UW solution at pH 6.8 or 6.4 resulted in poorer preservation of the dog kidney than at pH 7.4.

This study was based on reports [2, 15] that showed a beneficial effect of acidic pH in cytoprotection of isolated renal cells exposed to warm anoxic storage. We reasoned that pH and hypothermically induced metabolic suppression may, in combination, provide better kidney preservation than hypothermia alone. The fact that acidic storage conditions yielded worse results suggests that the mechanisms of warm, anoxia-induced injury may be different than those of cold anoxia-induced injury. The poorer results obtained by preservation at acidic pHs versus more alkaline conditions in this study may mean that vascular cells are more sensitive to acidic conditions than parenchymal cells since isolated parenchymal cells are not injured by acidic pH. Our results, therefore, suggest that unlike isolated cells, the whole organ is not protected by cold storage under acidic conditions but, in fact, show a preference for slightly alkaline conditions (pH 7.4–7.8).

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